



Nitrate Protocol



Purpose

To obtain nitrate nitrogen measurements of the water at the Hydrology Study Site

Overview

Measuring nitrate levels in water is an important step in the determination of water quality. Nitrogen exists in water in numerous forms, two of which are nitrate (NO_3^-) and nitrite (NO_2^-). Of these forms, nitrate is usually the most important. Nitrite can be found in suboxic waters. Nitrate is an essential nutrient for growth of algae and other aquatic plants, and can be present at high levels due to inputs from a variety of sources. Nitrate is very difficult to measure directly, so it is reduced to nitrite and the resulting nitrite concentration is measured. The measurement gives the combined concentration of nitrite (if present) and nitrate concentrations. Because we are interested in the nitrate measurement, background levels of nitrite also have to be measured. Nitrate measurements are reported as nitrate nitrogen (mg/L). Nitrite measurements are reported as nitrite nitrogen (mg/L).

Level

Intermediate and Advanced

Time

About 15 minutes

Frequency

Weekly

Calibration every six months

Key Concepts

Colorimetric methods for water analysis
Nitrate in water

Skills

Doing a colorimetric analysis
Designing measurement strategies
Recording data

Materials and Tools

50 mL beaker or flask
Nitrate Test Kit (if you have salt or brackish water, be sure to use an appropriate test kit)
100 mL graduated cylinder
500 mL graduated cylinder
3 500-mL bottles or jars
Distilled water

Preparation

Read all instructions carefully in the test kit before beginning. Make sure kit includes all the materials listed. Review proper levels of nitrate that are acceptable in water (10 mg/L nitrate-nitrogen for drinking water).

Prerequisites

A brief discussion of why nitrate is important in water

A discussion of the difference between nitrate nitrogen and nitrate

A discussion of the difference between nitrate and nitrite

Practice by doing calibration.

Calibration and Quality Control

Standards should be run at least every six months to verify your technique and the integrity of your chemicals. Fresh standard should be prepared each time unless the standard has been stabilized. Measuring the standards will help to clarify the instructions in test kits where wording may be unclear.

Nitrate Standards

Nitrate standards do not come with test kits and need to be either ordered separately or prepared as follows:

- Stock Nitrate Solution: Dry KNO_3 (potassium nitrate) in an oven for 24 hours at 105°C . Then dissolve 3.6 g of KNO_3 in distilled water. Dilute to 500 mL in your 500 mL graduated cylinder using distilled water. Carefully swirl the solution to mix (do not shake). Store in a 500 mL bottle or jar. Label with masking tape (include date). This makes a 7200 mg/L KNO_3 (or a 1000 mg/L nitrate nitrogen) solution.

Note: To calculate nitrate nitrogen ($\text{NO}_3\text{-N}$), take into account the molecular composition of KNO_3 (the ratio of the molecular weight of N to KNO_3 is 0.138): $7200\text{ mg/L } \text{KNO}_3 \times 0.138 \approx 1000\text{ mg/L nitrate nitrogen } (\text{NO}_3\text{-N})$.

- Standard Nitrate Solution: Measure 50 mL of the stock nitrate solution using the 100 mL graduated cylinder. Pour into the 500 mL graduated cylinder and dilute to 500 mL with distilled water. Carefully swirl the solution to mix. The result is a 100 mg/L nitrate nitrogen standard. Store in a 500 mL bottle or jar. Label with masking tape (include date).
- Make a new stock nitrate solution each time a calibration is conducted if the stock solution has not been preserved. Standard nitrate solutions should be made fresh each time regardless of whether the stock solution has been preserved or not. The stock nitrate solution can be preserved and stabilized for up to six months using chloroform (CHCl_3) if you

have safe access to this chemical. To preserve a stock nitrate standard add 1 mL of CHCl_3 to 500 mL of stock solution.

Quality Control Procedure

1. Dilute the 100 mg/L standard to make a 2 mg/L standard. Use this standard to test the accuracy of the nitrate kit. Measure out 10 mL of the 100 mg/L standard nitrate solution using the 100 mL graduated cylinder. Pour this into the 500 mL flask or beaker. Measure out 490 mL of distilled water in the 500 mL graduated cylinder and add to 500 mL bottle or jar. Label with masking tape (include date). Carefully swirl the solution to mix the standard.
2. Follow the directions in the *Protocol* section to measure the standard. Where it says “sample water” this is where you use the standard that you made.
3. Record the value of the standard after testing on the Hydrology Investigation Data Work Sheet.
4. If the nitrate standard is off by more than 1 mg/L, prepare new dilutions and repeat the measurement. If still off, make a new stock solution and repeat the procedure.

How to Measure Nitrate Nitrogen

1. Use a nitrate measurement kit that meets the *GLOBE Instrument Specifications* in the *Toolkit*. Rinse the sample tubes in the kit at least 3 times with sample water before starting the measurement.
2. Nitrate nitrogen plus nitrite nitrogen: Follow the manufacturer's nitrate instructions in the kit. The kits are based on the technique of adding a reagent that reacts with nitrate to form nitrite. The nitrite reacts with a second reagent to form a color. The intensity of the color is proportional to the amount of nitrate in the sample. The concentration is determined by comparing the sample color, after addition of reagents, to a color comparator included in the kit. If the kit calls for shaking the sample, be sure to shake for the specified period of time.



Failure to follow the times specified in the directions will result in inaccurate measurements.

3. Have at least 3 students in the group read the color comparator. Record the nitrate concentration for each student group on the Hydrology Investigation Data Work Sheet. (Note: Hold the comparator up to a light source such as a window, the sky or a lamp. Do not hold it up to the sun.)
4. Take the average of the three readings. If the recorded values are all within 1 mg/L of the average, record the average on the Hydrology Investigation Data Work Sheet. If they are not within 1 mg/L of the average have the students reread the color comparator, then record and average the new values. (Note: do not reread if more than 5 minutes has elapsed.) If your remaining values are now all within 1 mg/L of the new average, record this new average on the Hydrology Investigation Data Work Sheet. If there is still one outlier (a value far different from the rest) discard that value and calculate a new average of the other values. If there is still wide scatter (more than 1 mg/L) in results, discuss the procedure and the potential sources of error with the students, but do not report a value to the Data Server. Repeat the protocol to produce a reportable measurement.
5. Nitrite nitrogen: Follow the manufacturer's instructions for nitrite. It is the same procedure, except the reagent to reduce nitrate to nitrite is not used.
6. Repeat steps 3 and 4 to obtain nitrite values.

Note: Test results should be reported as mg/L nitrate nitrogen ($\text{NO}_3\text{-N}$; the same units as your standards), and not as mg/L nitrate (NO_3^-).

For general information: To convert mg/L nitrate to mg/L nitrate nitrogen divide by 4.4, the ratio of their molecular weights. For example: 44 mg/L NO_3^- is equivalent to 10 mg/L $\text{NO}_3\text{-N}$. To convert mg/L nitrite to mg/L nitrite nitrogen divide by 3.3, the ratio of their molecular weights.